Use of antiretroviral therapy and risk of end-stage liver disease and hepatocellular carcinoma in HIV-positive persons

Ryom, Lene ; Lundgren, Jens Dilling ; De Wit, Stéphane ; Kovari, Helen ; Reiss, Peter ; Law, Matthew ; El-Sadr, Wafa ; Monforte, Antonella D'Arminio ; Mocroft, Amanda ; Smith, Colette ; Fontas, Eric ; Dabis, Francois ; Phillips, Andrew ; Sabin, Caroline

Abstract: OBJECTIVES Although several antiretroviral drugs, including the d-drugs stavudine (d4T) and didanosine (ddI), may cause biomarker-defined hepatotoxicity, their association with clinically defined end-stage liver disease (ESLD) and hepatocellular carcinoma (HCC) remains unknown. DESIGN Prospective cohort study. METHODS Data collection on adverse events of anti-HIV drugs study (D:A:D) participants were followed until the first of ESLD (variceal bleeding, hepatic encephalopathy, hepatorenal syndrome or liver transplantation), HCC (histology or -fetoprotein along with imaging), death, 6 months after last visit or 1 February 2014. Associations between ESLD/HCC and cumulative use of individual antiretrovirals were investigated using Poisson regression adjusting for potential confounders. RESULTS During a median follow-up of 8.4 years, 319 ESLD/HCC cases occurred [incidence 1.01/1000 person-years (95% confidence interval 0.90-1.12)] with a 1-year mortality rate of 62.6%. After adjustment, cumulative (per 5 years) exposure to d4T [relative rate 1.46 (95% confidence interval 1.20-1.77)], ddI [1.32 (1.07-1.63)], tenofovir [TDF, 1.46 (1.11-1.93)] and (fos)amprenavir [APV; 1.47 (1.01-2.15)] was associated with increased ESLD/HCC rates. Longer exposure to emtricitabine [0.51 (0.32-0.83)] and nevirapine [0.76 (0.58-0.98)] were associated with lower ESLD/HCC rates. The ddI/d4T-associated increased ESLD/HCC rate only started to decline 6 years after cessation. CONCLUSION Cumulative use of d4T, ddI, TDF and APV were independently associated with increased ESLD/HCC rates, and intensified monitoring of liver function should hence be considered among all individuals exposed for longer time periods. The use of d-drugs should furthermore be avoided, where there are alternatives available, and focus should be put on those with longer-term d-drugs exposure who remain at increased ESLD/HCC risk. The unexpected, and viral hepatitis-independent, TDF association calls for further investigations.

DOI: https://doi.org/10.1097/QAD.0000000000001018

Use of antiretroviral therapy and risk of end-stage liver disease and hepatocellular carcinoma in HIV-positive persons

Lene Ryom[a], Jens Dilling Lundgren[a], Stéphane De Wit[b], Helen Kovari[c], Peter Reiss[d], Matthew Law[e], Wafa El-Sadr[f], Antonella D'Arminio Monforte[g], Amanda Mocroft[h], Colette Smith[h], Eric Fontas[i], Francois Dabis[j,k], Andrew Phillips[h], Caroline Sabin[h], for the D:A:D Study Group

See related paper on page 1843

Objectives: Although several antiretroviral drugs, including the d-drugs stavudine (d4T) and didanosine (ddI), may cause biomarker-defined hepatotoxicity, their association with clinically defined end-stage liver disease (ESLD) and hepatocellular carcinoma (HCC) remains unknown.

Design: Prospective cohort study.

Methods: Data collection on adverse events of anti-HIV drugs study (D:A:D) participants were followed until the first of ESLD (variceal bleeding, hepatic encephalopathy, hepatorenal syndrome or liver transplantation), HCC (histology or α-fetoprotein along with imaging), death, 6 months after last visit or 1 February 2014. Associations between ESLD/HCC and cumulative use of individual antiretrovirals were investigated using Poisson regression adjusting for potential confounders.

Results: During a median follow-up of 8.4 years, 319 ESLD/HCC cases occurred [incidence 1.01/1000 person-years (95% confidence interval 0.90–1.12)] with a 1-year mortality rate of 62.6%. After adjustment, cumulative (per 5 years) exposure to d4T [relative rate 1.46 (95% confidence interval 1.20–1.77)], ddI [1.32 (1.07–1.63)], tenofovir [TDF, 1.46 (1.11–1.93)] and (fos)amprenavir [APV; 1.47 (1.01–2.15)] was associated with increased ESLD/HCC rates. Longer exposure to emtricitabine [0.51 (0.32–0.83)] and nevirapine [0.76 (0.58–0.98)] were associated with lower ESLD/HCC rates. The ddI/d4T-associated increased ESLD/HCC rate only started to decline 6 years after cessation.

Conclusion: Cumulative use of d4T, ddI, TDF and APV were independently associated with increased ESLD/HCC rates, and intensified monitoring of liver function should hence be considered among all individuals exposed for longer time periods. The use of d-drugs should furthermore be avoided, where there are alternatives available, and

[a]Department of Infectious Diseases, CHIP, Section 8632, Finsenscentret, Rigshospitalet, University of Copenhagen, Copenhagen, Denmark, [b]Department of Infectious Diseases, CHU Saint-Pierre, Brussels, Belgium, [c]Division of Infectious Diseases and Hospital Epidemiology, University Hospital Zurich, University of Zurich, Switzerland, [d]Division of Infectious Diseases and Department of Global Health, Academic Medical Center, University of Amsterdam, and the HIV Monitoring Foundation, Amsterdam The Netherlands, [e]The Kirby Institute, UNSW, Sydney, Australia, [f]ICAP-Columbia University and Harlem Hospital, New York, USA, [g]Dipartimento di Scienze della Salute, Clinica di Malattie Infectitive e Tropicali, Azienda Ospedaliera-Polo Universitario San Paolo, Milan, Italy, [h]Research Department of Infection and Population Health, UCL, London, UK, [i]Department of Public Health, Nice University Hospital, Nice, France, [j]Université Bordeaux Segalen, INSERM U 897, and [k]Epidemiologie-Biostatistique, CHU de Bordeaux, France.

Correspondence to Lene Ryom, MD, PhD, Department of Infectious Diseases, CHIP, Rigshospitalet, Section 8632, Finsencentret, University of Copenhagen, Blegdamsvej 9, DK-2100 Copenhagen O, Denmark.

Tel: +45 35 45 57 65; fax: +45 35 45 57 57; e-mail: lene.ryom.nielsen@regionh.dk
Received: 7 August 2015; revised: 15 December 2015; accepted: 31 December 2015.

DOI:10.1097/QAD.0000000000001018

focus should be put on those with longer-term d-drugs exposure who remain at increased ESLD/HCC risk. The unexpected, and viral hepatitis-independent, TDF association calls for further investigations.

AIDS 2016, **30**:1731–1743

Keywords: (fos)amprenavir, d-drugs, end-stage liver disease, hepatocellular carcinoma, hepatotoxicity, HIV, tenofovir

Introduction

The majority of the antiretroviral drug classes used for the treatment of HIV have an intrinsic potential to cause hepatotoxicity, which may be induced through various mechanisms [1–4]. Previous studies have estimated that up to 30% of all HIV-positive persons will experience some form of hepatotoxicity associated with antiretroviral treatment (ART) [1–3]. Studies that have investigated adverse ART-liver effects have commonly been based on changes in liver-related biomarkers such as transaminases [3,5–9], fibrosis markers (e.g., hyaluronic acid) [10], imaging modalities or biopsy findings [11–17]. Furthermore, most studies have been of a relatively modest size or of a cross-sectional nature, making assessments of the time course between exposure and outcomes difficult [13–19]. Studies have also been primarily conducted among individuals who are coinfected with HIV and viral hepatitis, in whom the incidence of adverse hepatic impairment is elevated compared with HIV monoinfected individuals [5,14,18,20,21]. Indeed, a previous data collection on adverse events of anti-HIV drugs (D:A:D) study analysis in participant without viral hepatitis found that deaths related to liver failure are rare in HIV-positive individuals without viral hepatitis B (HBV) or C (HCV) [20].

The adverse effects of ART on development of liver impairment have been debated for years [20,22–25]. The diverse study outcomes are, however, not surprising as these will naturally depend on the individual antiretroviral investigated. ART is known to have an overall beneficial effect on liver-related outcomes, largely because of attenuating liver disease progression in viral hepatitis coinfected individuals by improvements in immune function [22,24], and the direct antiviral effect on HBV by certain individual antiretrovirals such as tenofovir (TDF), lamivudine (3TC) and emtricitabine (FTC). Conversely, other antiretrovirals, such as tipranavir (TPV), have a known direct hepatotoxic effect [4]. Furthermore, an excess risk of liver-related death with cumulative ART use has been observed after accounting for improvements in the $CD4^+$ cell count [24,26].

Among the individual antiretrovirals, dideoxynucleoside analogues or 'd-drugs' and, in particular, didanosine (ddI) and stavudine (d4T) have commonly been associated with alterations in liver function and severe steatosis/fibrosis development [11,13,16,17,27–31]. Moreover, ddI use has been linked with development of noncirrhotic portal hypertension [32,33]. Use of d-drugs is now rare in most high-income countries because of their serious adverse effects including neuropathy and lipodystrophy [4]. D-drug use was, however, common in the past, and previous exposure may also impact on subsequent liver function [34]. In 2010, the WHO launched an initiative aiming to reduce the use of d4T. However, many individuals in resource-limited settings continue to use d-drugs because of their widespread availability and low cost; as such, an estimated 1.1 million individuals still initiated a d4T-based first-line antiretroviral regimen in 2011 [35,36].

Of the nonnucleoside reverse transcriptase inhibitors (NNRTIs), in particular, nevirapine (NVP) has been linked to hepatotoxicity as part of a systemic hypersensitivity reaction [1,26,37–39]. Compared with the suspected liver steatosis/fibrosis effect of d-drugs and other nucleoside reverse transcriptase inhibitors (NRTIs), the NNRTI class effect on the liver is of a hepatitis-like nature [1,4,26,37–39].

Among the protease inhibitors (PIs), in particular, atazanavir (ATV) may cause hyperbilirubinemia and cholecystolithiasis, whereas TPV-related hepatitis is expected in 10% of recipients [4,40,41]. Coinfection with viral hepatitis has been associated with increased plasma levels of PIs, but the clinical impact of coinfection on antiretroviral-related hepatotoxicity is unknown [42].

Although most antiretroviral-related adverse liver effects are reported to be acute, mild and asymptomatic [3], prospective investigations of long-term antiretroviral use and clinical manifestations of end-stage liver disease (ESLD) and hepatocellular carcinoma (HCC) have not, as yet, been conducted in a large heterogeneous cohort setting. This analysis aimed to describe incidence rates, predictors and survival after ESLD/HCC with focus on the potential association with cumulative use of individual antiretrovirals, and, in particular, d-drugs, because of their high susceptibility to induce fibrosis.

Methods

Study population

The D:A:D study is a prospective cohort collaboration established in 1999 which follows more than 49 000 HIV-1-positive persons in Europe, the United States and Australia; details have been previously published [17]. Data on clinical events including ESLD, cancers, myocardial infarction, stroke, invasive cardiovascular procedures and death are collected in real time during routine clinical care. Events are validated centrally and monitored regularly. In addition, information on demographic factors, ART, viral hepatitis, laboratory test results, cardiovascular risk factors and AIDS events is electronically collected at enrolment and every 6 months.

Endpoint definition

From 1 February 2004 onwards, systematic collection of ESLD and cancer events (including HCC) was initiated in D:A:D. A designated ESLD event form was developed to collect detailed information on the clinical symptoms defining ESLD: bleeding from endoscopically verified gastric or oesophageal varices; stages III–IV hepatic encephalopathy; hepatorenal syndrome or liver transplantation (more information at www.chip.dk). In addition, pathology reports and transient elastographies results are collected. Information on HCC, including histology findings or the combination of elevated α-fetoprotein and HCC-specific imaging findings, is collected on a designated cancer form. Where a diagnosis of ESLD/HCC was reported only on a fatal case reporting form (Coding Causes of deaths in HIV, CoDe, more information at www.chip.dk) [43], the death was only considered to be an ESLD or HCC event if information was provided on the clinical symptoms and histology/imaging findings.

Statistical methods

D:A:D participants were followed from the date of enrolment in the study or 1 February 2004, if this was later (baseline), until the first of an ESLD/HCC event, death, 6 months after last visit or 1 February 2014. Only the first ESLD or HCC event reported for each individual during prospective follow-up was included in the analysis. Incidence rates were calculated per 1000 person-years of follow-up (PYFU). Kaplan–Meier estimation was used to describe the risk of mortality following a diagnosis of ESLD/HCC. Poisson regression models were used to quantify the relationship between ESLD/HCC and cumulative antiretroviral use. Potential confounders considered for adjusted models were calendar year (categorized as before 2008, 2008/2009 and after 2009), demographic variables [sex, age (<35 or >35 years)], ethnicity (white vs. other), participating cohort, smoking status (current, previous, never, unknown), HIV-related factors (route of HIV acquisition (IDU vs. other), previous AIDS diagnosis and HBV and HCV coinfection status (negative, positive, unknown), with variables retained in multivariable models if they were significantly associated ($P < 0.05$) with the outcome in univariate models.

Subsequent models also considered adjustment for the latest viral load (fitted categorically) and $CD4^+$ cell count, both as time-updated covariates; these covariates were purposely not included in our primary analyses of antiretroviral associations, as they may lie on the causal pathway between antiretroviral exposure and ESLD/HCC.

Antiretroviral exposure was fitted cumulatively (per 5 years) as it was hypothesized that the risk of any adverse liver events would increase with longer exposure [44]. All antiretroviral exposures were considered, regardless of when they accrued – thus, individuals may already have accrued several years of exposure to some of the antiretrovirals prior to baseline; exposure then continued to accrue after baseline if the individuals continued to receive the drugs (or restarted them at a later point in time).

An investigation of the association between time since d-drug discontinuation and ESLD/HCC rates was conducted adjusting for cumulative exposure to each drug and other potential confounders. This allowed us to assess the potential for any increased risk of ESLD/HCC to be reversed after cessation of the drug.

All statistical analyses were carried out using SAS version 9.3 (Statistical Analysis Software, Cary, North Carolina, USA).

Results

Characteristics

A total of 45 544 individuals were followed in D:A:D since 1 February 2004 and were included in the analysis; these individuals were predominantly white (49.6%), men (73.5%) and had acquired HIV through sex between men, MSM (44.5%) (Table 1). The median age was 40 [interquartile range (IQR) 34–46] years at baseline and the median $CD4^+$ cell count was 434 (IQR 282–621) cells/μl. During a median follow-up of 8.4 (IQR 5.5–12.6) years, a total of 319 ESLD/HCC events occurred (incidence rate 1.01/1000 PYFU, 95% confidence interval 0.90–1.12) of which 209 were ESLD and 110 HCC events. Overall, 157 (49.2%) of the ESLD/HCC events were identified through an ESLD form, 88 (27.6%) through a cancer form and the remaining 74 (23.2%) through a death form. The most common clinical manifestation of ESLD was hepatic encephalopathy grades III–IV (43.3%), followed by endoscopically verified variceal bleeding (27.4%), hepatorenal syndrome (14.6%) and liver transplantation (5.1%), whereas 9.6%

Table 1. Characteristics of persons included in analysis at baseline and at the time of end-stage liver disease/hepatocellular carcinoma (ESLD/HCC).

		All eligible participants at baseline	At the time of an ESLD/HCC event
Number of participants		45 544 (100.0)	319 (100.0)
Sex	Men	33 465 (73.5)	254 (79.6)
	Women	12 079 (26.5)	65 (20.4)
Age (years)	Median (IQR)	40 (34–46)	47 (42–52)
Mode of acquisition	MSM	20 284 (44.5)	64 (20.1)
	IDU	6396 (14.0)	171 (53.6)
	Heterosexual	15 316 (33.6)	67 (21.0)
	Other/unknown	3548 (7.8)	17 (5.3)
Ethnicity	White	22 609 (49.6)	180 (56.4)
	Black African	4263 (9.4)	12 (3.8)
	Other	1265 (2.8)	1 (0.3)
	Unknown	17 407 (38.2)	126 (39.5)
$CD4^+$ cell count (cells/μl)	Median (IQR)	434 (282–621)	266 (153–448)
HIV RNA (log_{10} copies/ml)	Median (IQR)	2.3 (1.7–4.3)	1.7 (1.7–2.6)
HCV status[a]	Negative	29 002 (63.7)	71 (22.3)
	Positive	8259 (18.1)	231 (72.4)
	Unknown	8283 (18.2)	17 (5.3)
HBV status[b]	Negative/Not active	36711 (80.6%)	238 (74.6%)
	Positive active	2074 (4.6)	63 (19.8)
	Unknown	6759 (14.8)	18 (5.6)
Smoking status	Current smoker	17 443 (38.7)	180 (56.4)
	Ex-smoker	7682 (17.0)	84 (26.3)
	Never smoker	11 909 (26.4)	43 (13.5)
	Not known	8089 (17.9)	12 (3.8)
Previous AIDS		10 846 (23.8)	155 (48.6)

[a]HCV status (negative: seronegative, or seropositive but HCV-RNA negative; positive: seropositive and HCV-RNA positive or HCV-RNA unknown or not tested).
[b]HBV status [positive: active infection (HBs antigen, HBe antigen, or HBV-DNA positive).

had more than one manifestation at the same event date. The median age of individuals experiencing ESLD/HCC (at the time of the event) was 47 (IQR 42–52) years, the most common mode of HIV acquisition was IDU (53.6%), the median $CD4^+$ cell count was 266 (IQR 153–448) cells/μl and 72.4% were confirmed HCV positive and 19.8% with active HBV co-infection (Table 1). As 82.8% of all events occurred in individuals coinfected with viral hepatitis, the ESLD/HCC incidence rate was low in individuals without HCV or HBV evidence [0.12/1000 PYFU (95% confidence interval 0.07–0.16)] (Table 2). Although the ESLD/HCC incidence rate was similar for individuals with HIV/HCV and HIV/HBV coinfection, the number of individuals with HIV/HCV/HBV was limited (data not shown).

Prognosis

The 319 individuals with ESLD/HCC were followed for a median of 0.23 (IQR 0.01–1.88) years after diagnosis, over which time 241 (75.6%) died. The median survival after an ESLD/HCC diagnosis was 0.27 years, whereas the 1-year mortality rate (Kaplan–Meier estimate) was 62.6%. After exclusion of 52 individuals diagnosed with ESLD/HCC at time of death, the median survival after an

Table 2. Incidence of end-stage liver disease/hepatocellular carcinoma (per 1000 person-years of follow-up) stratified by viral hepatitis status.

Factor		No. of events	PYFU	Rate	95% CI
Overall		319	315 368	1.01	0.90–1.12
HCV status[a]	Negative	72	229 434	0.31	0.24–0.39
	Positive	229	63 786	3.59	3.13–4.06
	Unknown	18	22 148	0.81	0.48–1.28
HBV status[b]	Negative/Not active	240	284917	0.84	0.74-0.95
	Positive active	59	12 907	4.57	3.40–5.74
	Unknown	20	17 545	1.14	0.64–1.64

CI, confidence interval; HBV, hepatitis B virus; HCV, hepatitis C virus.
[a]HCV status (negative: seronegative, or seropositive but HCV-RNA negative; positive: seropositive and HCV-RNA positive or HCV-RNA unknown or not tested).
[b]HBV status positive: active infection (HBs antigen, HBe antigen, or HBV-DNA positive).

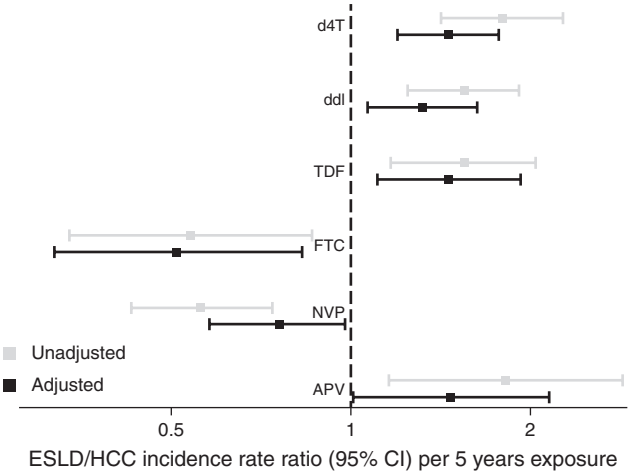

Fig. 1. Association between cumulative (per 5 year) antiretroviral exposure and end-stage liver disease/hepatocellular carcinoma (ESLD/HCC). APV, (fos)amprenavir; d4T, stavudine; ddI, didanosine; FTC, emtricitabine; NVP, nevirapine; TDF, tenofovir.

ESLD/HCC event was 0.66 years and the 1-year mortality rate was 55.0%.

Associations between antiretroviral exposure and end-stage liver disease and hepatocellular carcinoma

Associations between individual antiretrovirals and ESLD/HCC were initially explored without adjustment for calendar year, demographics, HIV-related factors and viral hepatitis status (but with adjustment for other antiretrovirals in the regimen) (Fig. 1).

Among the NRTIs a linear and similar cumulative effect of ddI and d4T was observed [d4T 1.80/5 years (1.42–2.27) and ddI 1.55/5 years (1.25–1.92)]. Increased exposure to 3TC [1.31/5 years (1.07–1.60)] and TDF [1.55/5 years (1.17–2.04)] were unexpectedly also associated with increased rates of ESLD/HCC. In contrast, a reduced rate of ESLD/HCC was seen in individuals with longer exposure to FTC [0.54/5 years (0.34–0.86)]. After adjustment for potential confounders, significant associations remained with cumulative use of d4T [1.46/5 years (1.20–1.77)], ddI [1.32/5 years (1.07–1.63)], TDF [1.46/5 years (1.11–1.93)] and FTC [0.51/5 years (0.32–0.83)], whereas the association with 3TC no longer remained significant (Fig. 1).

Among the NNRTIs, use of NVP was surprisingly associated with a reduced rate of ESLD/HCC in both unadjusted [0.56/5 years (0.43–0.74)] and adjusted [0.76/5 years (0.58–0.98)] analyses.

(Fos)amprenavir (APV) was the only PI associated with ESLD/HCC in unadjusted models [1.82/5 years (1.16–2.85)] and this association remained significant in the fully adjusted multivariate model [1.47/5 years (1.01–2.15)].

A sensitivity analysis stratified according to viral hepatitis status reached consistent associations for all antiretrovirals, including TDF, although the number of events was low in those without evidence of HBV/HCV (data not shown).

The associations with ddI and d4T were explored in more detail, as determined *a priori* because of the potential long-term effects on liver function after ceased use. When exposure to ddI was considered without any d4T exposure, the association between ddI and ESLD/HCC was slightly reduced [1.28/5 years (0.98–1.66)] as was d4T exposure when used without ddI [1.43/5 years (1.16–1.77)]. When used concomitantly, the association with ESLD/HCC was strengthened [2.03/5 years (1.44–2.85)], however, only to the extent that would be expected on the basis of combining the effects of each drug when used separately, as there was no evidence of synergy between the two drugs in this model. There was no strong evidence to indicate that the associations between the two d-drugs and ESLD/HCC differed between individuals with and without viral hepatitis coinfection ($P = 0.50$ for interaction between d4T and any hepatitis, $P = 0.09$ for ddI and any hepatitis), although because of the small number of events in those without coinfection, the power to detect a significant interaction was extremely low.

Of the 18 676 persons on d4T or ddI, 91.4% stopped their use at least once during follow-up, with only 18.4% of the PYFU in those exposed to d-drugs being current users. Those having previously stopped d-drugs had higher ESLD/HCC rates than those currently on d-drugs, an effect that only started to wane slightly after more than 6 years after cessation (Table 3).

Discussion

This is the first large, prospective and long-term analysis to investigate incidence, outcomes and risk factors for clinically defined liver failure and cancer in HIV-positive persons with focus on the contribution of individual antiretrovirals.

We observed a relatively low overall incidence rate of ESLD/HCC in this large heterogeneous cohort of both HIV monoinfected and viral hepatitis coinfected persons. In comparison, a recent retrospective analysis from the Veterans Affairs cohort found that among antiretroviral-treated HIV/HCV coinfected persons, 7.4% experienced hepatic decompensation at 10 years [45]. This higher rate may, however, be explained by the conservative ESLD/HCC criteria in D:A:D, and by the inclusion of ascites in the definition of hepatic decompensation, which was not originally included in the D:A:D definition as it may also be seen in other noncirrhotic liver disease-related conditions [46].

Table 3. Associations between current, cumulative and past exposure to d-drugs (ddI and d4T) and rates of end-stage liver disease/ hepatocellular carcinoma (ESLD/HCC).

		No other factor	Adjusted for exposure to other NRTIs, PIs and NNRTIs	Exposure to other NRTIs, PIs and NNRTIs and potential confounders[c]
	Rate of ESLD/HCC (per 1000 PYFU[a], 95% CI[a])	Relative rate[b] (95% CI)	Relative rate (95% CI)	Relative rate (95% CI)
Never received d-drugs	0.50 (0.40, 0.61)	0.60 (0.37, 0.98)	0.65 (0.40, 1.05)	1.03 (0.60, 1.73)
Currently on d-drugs	1.30 (0.87, 1.76)	Ref.	Ref.	Ref.
Stopped d-drugs and off for:				
≥0, <2 years	1.89 (1.32, 2.45)	1.70 (1.07, 2.69)	1.72 (1.08, 2.72)	1.64 (1.03, 2.60)
≥2, <4 years	1.85 (1.30, 2.41)	1.60 (1.01, 2.52)	1.65 (1.04, 2.61)	1.59 (1.00, 2.52)
≥4, <6 years	1.93 (1.36, 2.50)	1.63 (1.04, 2.56)	1.72 (1.09, 2.73)	1.63 (1.03, 2.59)
≥6, <8 years	1.56 (1.00, 2.12)	1.34 (0.81, 2.20)	1.48 (0.89, 2.46)	1.48 (0.89, 2.47)
≥8 years	1.40 (0.95, 1.85)	1.25 (0.78, 2.01)	1.44 (0.88, 2.36)	1.49 (0.90, 2.47)
Cumulative exposure (/year) to d-drugs	n/a	1.07 (1.03, 1.11)	1.07 (1.03, 1.12)	1.06 (1.01, 1.10)

[a]CI, confidence interval; d4T, stavudine; ddI, didanosine; NNRTIs, nonnucleoside reverse transcriptase inhibitors; NRTIs, nucleoside reverse transcriptase inhibitors; PIs, protease inhibitors; PYFU, person-years of follow-up.
[b]Adjusted for time since stopping d-drug and cumulative exposure to d-drug.
[c]Age, sex, injection drug use as mode of HIV acquisition, previous AIDS diagnosis, HBV, HCV, calendar period, time since stopping d-drug and cumulative exposure to d-drugs.

The prognosis following ESLD/HCC was poor with a median survival of only 0.27 years. This observation calls for an increased awareness of ESLD/HCC risk factors and management. The recent introduction of effective direct-acting agents (DAAs) for treatment of HCV will likely change the ESLD/HCC incidence and survival over the years to follow.

Antiretroviral risk factors of end-stage liver disease and hepatocellular carcinoma

We identified cumulative use of ddI, d4T, TDF and APV to be independently associated with an increased rate of ESLD/HCC development, whereas use of FTC and NVP were associated with decreased rates.

The association between cumulative ddI and d4T use and excess ESLD/HCC incidence builds on the observations of a number of relatively small studies of HIV-monoinfected and viral hepatitis coinfected individuals using various biomarkers of liver failure [5,14,18]. A recent retrospective study among 146 HIV/HCV coinfected persons found that each additional year of use of these drugs was associated with a 50% increase in the odds of progressing one or more grades on the Brunt score [15]. Similarly, a subanalysis among 205 HIV/HCV coinfected persons randomized to two types of anti-HCV treatment found that ddI use was associated with three-fold higher odds of histologically verified fibrosis [16], and a European cross-sectional study of 671 HIV/HCV coinfected persons found that a median use of ddI exceeding 5 months increased the odds of severe liver fibrosis by 70% [13].

The incidence rate of ESLD/HCC among those who had been exposed to ddI and/or d4T was higher among individuals who had discontinued the drugs, than among those currently receiving them. This may reflect the fact that those at highest underlying risk of ESLD/HCC may be most likely to stop the d-drugs. Several mechanisms have been suggested for d-drug hepatotoxicity including inhibition of the mitochondrial DNA polymerase gamma and the mitochondrial respiratory chain with resulting oxidative damages and lactic acidosis [3,16,47,48], hepatic steatosis (microvascular and macrovascular), hepatocellular damage and ultimately development of cirrhosis [13,15].

The observed higher incidence of ESLD/HCC did not begin to decrease until 6 years after cessation of ddI and d4T use, suggesting that exposure to the drugs may have caused irreversible tissue damage. This finding is in accordance with work from Scourfield *et al.* [34] who found that the adverse liver effects of ddI developed late and after use of the drug was discontinued. These observations hence have important implications for the clinical management of all HIV-positive persons with current or prior d-drug use. Use of d-drugs should therefore be avoided if possible, in particular in individuals with high underlying risk of ESLD/HCC such as those with viral hepatitis. Owing to the long-lasting adverse effects of d-drugs also after their use have been discontinued one might further consider intensifying monitoring with liver biomarkers, and if abnormal, by transient elastography or liver biopsy among individuals with long-term prior d-drug use to better identify individuals at increased ESLD/HCC risk.

In contrast to the associations seen with ddI and d4T, the observed association between ESLD/HCC and TDF was unexpected. As TDF may be used preferentially among those with HBV coinfection, it may not be surprising that we see an increased rate of hepatotoxicity in those exposed to this drug [1,49]. Importantly, however, the TDF association remained unchanged after stratifying

according to and adjusting for viral hepatitis status, suggesting the association is not dependent on HBV and is not simply explained by an increased ESLD/HCC risk among individuals coinfected with HBV and preferentially treated with TDF. Furthermore, a recent D:A:D analysis which investigated predictors of chronic liver enzyme elevation among individuals without viral hepatitis also confirmed the positive association with cumulative TDF use, supporting this observation [50]. Finally 3TC, which is also used to treat HBV infection, although less often because of a lower genetic resistance barrier, did not remain statistically significantly associated with ESLD/HCC in adjusted models. Use of FTC, which is commonly coprescribed with TDF (68% of those currently on TDF were also on FTC) was further independently associated with a lower ESLD/HCC risk and further argues against the hypothesis that our observed TDF association simply reflects a higher rate of unreported HBV infection. Outside the D:A:D study, only a few studies, predominantly smaller case reports, have reported a positive association between liver impairment and TDF [49,51,52]. This TDF association does, however, seem to be robust in D:A:D and is related to several liver outcomes, calling for confirmation in other large studies. No biological mechanism is currently known for TDF to cause ESLD/HCC, but the effect may relate to the development of mitochondrial toxicity in hepatocytes as described in renal tubular cells [53], and steatosis is described in the TDF product information [54]. Our results also call for further investigations in mechanistic studies.

Based on the literature, if anything, one would have expected use of NVP to be associated with increased risk of ESLD/HCC [1,26,37–39]. Instead, we observed a lower rate of ESLD/HCC associated with cumulative NVP use. This may reflect the fact that NVP may only contribute to acute, but not more advanced and chronic stages of liver disease, but may also reflect some degree of confounding by indication with NVP not being prescribed to high-risk individuals, or being discontinued in those who experience liver enzyme elevations after starting the drug.

Elevated levels of transaminases are a common adverse effect of APV, although a recent small study did not identify a safety concern with long-term use [55,56]. A recent mechanistic study further found some evidence to support an anti-HCC effect of APV [57]. APV may, because of its use in calculating dose recommendations for various levels of liver impairment, preferably have been used in individuals with liver impairment and the use is currently limited [58]. No other statistically significant associations were observed for cumulative use of other protease inhibitors including ATV. This suggests that from a clinical liver endpoint perspective, the commonly used PIs can be safely used in HIV-positive individuals over longer periods of time, a finding that is in accordance with other recent studies using biomarker-defined hepatotoxicity. Of note, use of TPV and darunavir are still too limited to allow for robust statistical analyses in D:A:D. Furthermore, our analysis did not consider any impact of DAAs for treatment of HCV, where there are known drug–drug interactions with PIs, as the use of DAAs in D:A:D is still extremely low.

Limitations

The limitations of this analysis should be acknowledged and include the lack of a systematic collection on information on alcohol consumption. However, the associations observed with use of ddI, d4T, TDF, APV, NVP and FTC are unlikely to be confounded by alcohol usage, as the choice of ART including these antiretrovirals is unlikely to be modified by the clinician's knowledge of the individuals alcohol consumption. A high number of antiretrovirals were included in the analysis, hence we cannot rule out that findings may be a result of multiple testing and the possibility of false-positive errors. Confounding by indication cannot be ruled out for a number of the included antiretrovirals, in particular, as discussed, APV and NVP. Finally, nonantiretroviral hepatotoxic treatment, such as antituberculosis treatment with isoniazid and rifampicin, or sulphonamides used to treat pneumocystis pneumoni, may represent unmeasured confounding although adjustment for previous AIDS events did not change any of the observed antiretroviral associations.

Conclusion

While the ESLD/HCC incidence was relatively low in this large heterogeneous cohort, and predominantly seen in individuals with viral hepatitis coinfection, the prognosis following a diagnosis was very poor. Cumulative use of ddI, d4T, TDF and APV was independently associated with increased rates of ESLD/HCC, whereas use of NVP and FTC was associated with lower rates. There was limited evidence for reversibility of ESLD/HCC risk upon cessation of d-drugs, and intensified monitoring of liver function and avoidance of hepatotoxic compounds should hence be considered among all with longer-term current or prior d-drug exposure. The unexpected, and viral hepatitis independent, TDF association calls for further investigations, whereas use of d-drugs should be avoided, where there are alternatives available.

Acknowledgements

Funding: This work was supported by the Highly Active Antiretroviral Therapy Oversight Committee (HAART-OC), a collaborative committee with representation from academic institutions, the European Agency for the Evaluation of Medicinal Products, the United States Food and Drug Administration, the patient community and all

pharmaceutical companies with licensed anti-HIV drugs in the European Union: AbbVie, Boehringer Ingelheim, Bristol-Myers Squibb, Gilead Sciences, ViiV Healthcare, Merck, Pfizer, F. Hoffman-LaRoche and Janssen Pharmaceuticals.

Supported by a grant (grant number CURE/97-46486) from the Health Insurance Fund Council, Amstelveen, the Netherlands, to the AIDS Therapy Evaluation Project Netherlands (ATHENA); by a grant from the Agence Nationale de Recherches sur le SIDA (grant number Action Coordonnée no.7, Cohortes), to the Aquitaine Cohort; The Australian HIV Observational Database (AHOD) is funded as part of the Asia Pacific HIV Observational Database, a program of the Foundation for AIDS Research, amfAR, and is supported in part by a grant from the US National Institutes of Health's National Institute of Allergy and Infectious Diseases (NIAID) (grant number U01-AI069907) and by unconditional grants from Merck; Gilead Sciences; Bristol-Myers Squibb; Boehringer Ingelheim; F. Hoffman-LaRoche; Pfizer; GlaxoSmithKline and Janssen Pharmaceuticals. The Kirby Institute is funded by the Australian Government Department of Health and Ageing, and is affiliated with the Faculty of Medicine, the University of New South Wales. By grants from the Fondo de Investigación Sanitaria (grant number FIS 99/0887) and Fundación para la Investigación y la Prevención del SIDA en Espanã (grant number FIPSE 3171/00), to the Barcelona Antiretroviral Surveillance Study (BASS); by the National Institute of Allergy and Infectious Diseases, National Institutes of Health (grants number 5U01AI042170-10, 5U01AI046362-03), to the Terry Beirn Community Programs for Clinical Research on AIDS (CPCRA); by grants from the BIOMED 1 (grant number CT94-1637) and BIOMED 2 (grant number CT97-2713) programs and the fifth framework program (grant number QLK2-2000-00773) of the European Commission and grants from Bristol-Myers Squibb, GlaxoSmithKline, Boehringer Ingelheim and Roche, to the EuroSIDA study; by unrestricted educational grants of AbbVie, Bristol-Myers Squibb, Gilead Sciences, Glaxo-SmithKline, Pfizer and Janssen Pharmaceuticals to the Italian Cohort Naive to Antiretrovirals (the ICONA Foundation) and by a grant from the Swiss National Science Foundation, to the Swiss HIV Cohort Study (SHCS).

The content of this publication is solely the responsibility of the authors and does not necessarily represent the official views of any of the institutions mentioned above.

D:A:D participating cohorts: AHOD (Australia), Aquitaine (France), Athena (The Netherlands), BASS (Spain), CPCRA (USA), EuroSIDA (multinational), HivBivus (Sweden), ICONA (Italy), Nice (France), SHCS (Switzerland) and St. Pierre (Belgium).

D:A:D Steering Committee: Names marked with*, Chair with #Members of the D:A:D SC from the Oversight Committee: B. Powderly*, N. Shortman*, C. Moecklinghoff*, G. Reilly*, X. Franquet* D:A:D Central Coordination: L. Ryom, C.A. Sabin*, D. Kamara, C. Smith, A. Phillips*, A. Mocroft, A. Bojesen, J. Nielsen, D. Raben, J.D. Lundgren#; D:A:D data managers: R. Salbøl Brandt (coordinator), M. Rickenbach, I. Fanti, E. Krum, M. Hillebregt, S. Geffard, A. Sundström, M. Delforge, E. Fontas, F. Torres, H. McManus, S. Wright, J. Kjær. Verification of Endpoints: A. Sjøl (CVD primary endpoint), P. Meidahl (oncology), J. Helweg-Larsen (hematology), J. Schmidt Iversen (nephrology); Kidney working group: L. Ryom, A. Mocroft, O. Kirk*, P. Reiss*, M. Ross, C.A. Fux, P. Morlat, O. Moranne, D.A. Kamara, C. Smith, J.D. Lundgren#; Mortality working group: C. Smith, L. Ryom, A. Phillips*, R. Weber*, P. Morlat, C. Pradier*, P. Reiss*, N. Friis-Møller, J. Kowalska, J.D. Lundgren#; Cancer working group: C. Sabin*, M. Law*, A. d'Arminio Monforte*, F. Dabis*, M. Bruyand, P. Reiss*, C. Smith, D.A. Kamara, M. Bower, G. Fätkenheuer, A. Donald, A. Grulich, L. Ryom, J.D. Lundgren.

The members of the 11 Cohorts are as follows: ATHENA (AIDS Therapy Evaluation Project Netherlands): Central coordination: P. Reiss*, S. Zaheri, M. Hillebregt, L. Gras; Participating physicians (†Site coordinating physicians): Academisch Medisch Centrum bij de Universiteit van Amsterdam, Amsterdam: Prof. Dr J.M. Prins†, Prof. Dr T.W. Kuijpers, Dr H.J. Scherpbier, Dr J.T.M. van der Meer, Dr F.W.M.N. Wit, Dr M.H. Godfried, Prof. Dr. P. Reiss*,†, Prof. Dr T. van der Poll, Dr F.J.B. Nellen, Prof. Dr J.M.A. Lange, Dr S.E. Geerlings, Dr M. van Vugt, Dr D. Pajkrt, Dr J.C. Bos, Dr. M. van der Valk, Dr. M.L. Grijsen, Dr W.J. Wiersinga, Dr A. Goorhuis, Dr J.W.R. Hovius; Academisch Ziekenhuis Maastricht, Maastricht: Dr S. Lowe†, Dr A. Oude Lashof, Dr D. Posthouwer; Catharina-ziekenhuis, Eindhoven: Dr M.J.H. Pronk†, Dr H.S.M. Ammerlaan; Erasmus Medisch Centrum, Rotterdam: Dr M.E. van der Ende†, Dr T.E.M.S. de Vries-Sluijs, Dr C.A.M. Schurink, Dr J.L. Nouwen, Dr A. Verbon, Dr B.J.A. Rijnders, Dr E.C.M. van Gorp, Dr M. van der Feltz; Erasmus Medisch Centrum–Sophia, Rotterdam: Dr G.J.A. Driessen, Dr A.M.C. van Rossum; Flevoziekenhuis, Almere: Dr J. Branger†; HagaZiekenhuis, Den Haag: Dr E.F. Schippers†, Dr C. van Nieuwkoop, Dr. E.P. van Elzakker; Isala Klinieken, Zwolle: Dr P.H.P. Groeneveld†, Dr. J.W. Bouwhuis; Kennemer Gasthuis: Dr R. Soetekouw†, Prof. Dr R.W. ten Kate; Leids Universitair Medisch Centrum, Leiden: Dr F.P. Kroon†, Prof. Dr J.T. van Dissel, Dr S.M. Arend, Dr M.G.J. de Boer, Dr H. Jolink, Dr H.J.M. ter Vollaard, Dr M.P. Bauer; Maasstadziekenhuis, Rotterdam: Dr J.G. den Hollander†, Dr K. Pogany; Medisch Centrum Alkmaar, Alkmaar: Dr G. van Twillert†, Dr W. Kortmann†, Dr J.W.T. Cohen Stuart, Dr B.M.W. Diederen; Medisch Centrum Haaglanden, Den Haag:

Dr E.M.S. Leyten†, Dr L.B.S. Gelinck; Medisch Spectrum Twente, Enschede: Dr G.J. Kootstra†, Dr C.E. Delsing; Onze Lieve Vrouwe Gasthuis, Amsterdam: Prof. Dr K. Brinkman†, Dr W.L. Blok, Dr P.H.J. Frissen, Dr W.E.M. Schouten, Dr G.E.L. van den Berk; Sint Elisabeth Ziekenhuis, Tilburg: Dr M.E.E. van Kasteren†, Dr A.E. Brouwer; Sint Lucas Andreas Ziekenhuis, Amsterdam: Dr J. Veenstra†, Dr K.D. Lettinga; Sloter-vaartziekenhuis, Amsterdam: Dr J.W. Mulder†, Dr S.M.E. Vrouenraets, Dr F.N. Lauw; Stichting Medisch Centrum Jan van Goyen, Amsterdam: Dr A. van Eeden†, Dr D.W.M. Verhagen; Universitair Medisch Centrum Groningen, Groningen: Dr H.G. Sprenger†, Dr R. Doedens, Dr E.H. Scholvinck, Dr S. van Assen, Dr W.F.W. Bierman; Universitair Medisch Centrum Sint Radboud, Nijmegen: Dr P.P. Koopmans†, Dr M. Keuter, Dr A.J.A.M. van der Ven, Dr H.J.M. ter Hofstede, Dr A.S.M. Dofferhoff, Dr A Warris, Dr R. van Crevel; Universitair Medisch Centrum Utrecht, Utrecht: Prof. Dr A.I.M. Hoepelman†, Dr T. Mudrikova, Dr M.M.E. Schneider, Dr P.M. Ellerbroek, Dr J.J. Oosterheert, Dr J.E. Arends, Dr M.W.M. Wassenberg, Dr R.E. Barth; Vrije Universiteit Amsterdam, Amsterdam: Dr M.A. van Agtmael†, Dr R.M. Perenboom, Dr F.A.P. Claessen, Dr M. Bomers, Dr E.J.G. Peters; Wilhelmina Kinderzie-kenhuis, Utrecht: Dr S.P.M. Geelen, Dr T.F.W. Wolfs, Dr L.J. Bont; Ziekenhuis Rijnstate, Arnhem: Dr C. Richter†, Dr J.P. van der Berg, Dr E.H. Gisolf; Admiraal De Ruyter Ziekenhuis, Vlissingen: Dr M. van den Berge†, Dr A. Stegeman; Medisch Centrum Leeuwarden, Leeuwarden: Dr M.G.A. van Vonderen†, Dr D.P.F. van Houte; Medisch Centrum Zuiderzee, Lelystad: Dr S. Weijer†, Dr R. el Moussaoui; Sint Elisabeth Hospitaal, Willemstad – Curaçao: Dr C. Winkel, Dr F. Muskiet, Dr Durand, Dr R. Voigt.

Aquitaine Cohort (France): Principal investigator: Prof. F. Dabis*. Scientific committee: Prs F. Bonnet, F. Dabis*, M. Dupon, G. Chêne, D. Breilh, H. Fleury, D. Malvy, P. Mercié, I. Pellegrin, P. Morlat, D. Neau, JL. Pellegrin; Drs S. Bouchet, V. Gaborieau, D. Lacoste, S. Tchamgoué, R. Thiébaut. Composition of the GECSA: Epidemiol-ogy and biostatistics: Profs G. Chêne, F. Dabis, R. Thiébaut, Drs M. Bruyand, S. Lawson-Ayayi, L. Wittkop; Clinical and biological hospital units: Bordeaux University Hospital: Prof. P. Morlat (Prof. F. Bonnet, Drs N. Bernard, M. Hessamfar, D. Lacoste, MA. Vanden-hende); Prof. M. Dupon (Drs F.A. Dauchy, H. Dutronc), Prof. M. Longy-Boursier (Prof. P. Mercié, Drs P. Duffau, J. Roger Schmeltz), Prof. D. Malvy (Drs T. Pistone, M.C. Receveur), Prof. D. Neau (Drs C. Cazanave, A. Ochoa, M.O. Vareil), Prof. J.L. Pellegrin (Prof. J.F. Viallard, Drs C. Greib, E. Lazaro); Prof. H. Fleury (Prof. M.E. Lafon, Drs S. Reigadas, P. Trimoulet); Prof. D. Breilh; Prof. M. Molimard (Drs S. Bouchet, K. Titier); Prof. J.F. Moreau (Dr I. Pellegrin); Drs F. Haramburu, G. Miremont-Salamé. Arcachon Hospital: Dr A. Dupont. Dax Hospital: Dr Y. Gerard (Drs L. Caunègre, K. André). Bayonne Hospital: Dr F. Bonnal (Drs S. Farbos, M.C. Gemain). Libourne Hospital: Dr J. Ceccaldi (Dr S. Tchamgoué). Mont-de-Marsan Hospital: Dr S. De Witte (Dr C. Courtault). Pau Hospital: Dr E. Monlun (Dr V. Gaborieau). Périgueux Hospital: Dr P. Lataste (Dr J.P. Meraud). Villeneuve-sur-Lot Hospital: Dr I. Chossat. Permanent team: M.J. Blaizeau, M. Bruyand, V. Conte, M. Decoin, J. Delaune, S. Delveaux, F. Diarra, C. D'Ivernois, A. Frosch, S. Geffard, C. Hannapier, S. Lawson-Ayayi, E. Lenaud, O. Leleux, F. Le Marec, J. Leray, I. Louis, G. Palmer, A. Pougetoux, X. Sicard, D. Touchard, B. Uwamaliya-Nziyumvira.

AHOD (Australian HIV Observational Database, Aus-tralia): Central coordination: M. Law*, K. Petoumenos, H. McManus, S. Wright, C. Bendall (Sydney, New South Wales); Participating physicians (city, state): R. Moore, S. Edwards, J. Hoy, K. Watson, N. Roth, J. Nicholson (Melbourne, Victoria); M. Bloch, T. Franic, D. Baker, R. Vale, A. Carr, D. Cooper (Sydney, New South Wales); J. Chuah, M. Ngieng (Gold Coast, Queensland), D. Nolan, J. Skett (Perth, Western Australia).

BASS (Spain): Central coordination: G. Calvo*, F. Torres, S. Mateu (Barcelona); Participating physicians (city): P. Domingo, M.A. Sambeat, J. Gatell, E. Del Cacho, J. Cadafalch, M. Fuster (Barcelona); C. Codina, G. Sirera, A. Vaqué (Badalona).

The Brussels St Pierre Cohort (Belgium): Coordination: S. De Wit*, N. Clumeck, M. Delforge, C. Necsoi. Participating physicians: N. Clumeck, S. De Wit*, A.F. Gennotte, M. Gerard, K. Kabeya, D. Konopnicki, A. Libois, C. Martin, M.C. Payen, P. Semaille, Y. Van Laethem.

CPCRA (USA): Central coordination: J. Neaton, G. Bartsch, W.M. El-Sadr*, E. Krum, G. Thompson, D. Wentworth; Participating physicians (city, state): R. Luskin-Hawk (Chicago, Illinois); E. Telzak (Bronx, New York); W.M. El-Sadr (Harlem, New York); D.I. Abrams (San Francisco, California); D. Cohn (Denver, Colorado); N. Markowitz (Detroit, Michigan); R. Arduino (Houston, Texas); D. Mushatt (New Orleans, Louisiana); G. Friedland (New Haven, Connecticut); G. Perez (Newark, New Jersey); E. Tedaldi (Philadelphia, Pennsylvania); E. Fisher (Richmond, Virginia); F. Gordin (Washington, DC); L.R. Crane (Detroit, Michigan); J. Sampson (Portland, Oregon); J. Baxter (Camden, New Jersey).

EuroSIDA (multinational) Coordinating Centre: J. Lundgren*,#, O. Kirk*, A. Mocroft, A. Cozzi-Lepri, D. Grint, D. Podlekareva, J. Kjær, L. Peters, J. Reekie, J. Kowalska, J. Tverland, A.H. Fischer, J. Nielsen Partici-pating countries and physicians: Argentina: (M. Losso), C. Elias, Hospital J.M. Ramos Mejia, Buenos Aires. Austria: (N. Vetter), Pulmologisches Zentrum der Stadt

Wien, Vienna; R. Zangerle, Medical University Innsbruck, Innsbruck. Belarus: (I. Karpov), A. Vassilenko, Belarus State Medical University, Minsk, V.M. Mitsura, Gomel State Medical University, Gomel; O. Suetnov, Regional AIDS Centre, Svetlogorsk. Belgium: (N. Clumeck), S. De Wit*, M. Delforge, Saint-Pierre Hospital, Brussels; R. Colebunders, Institute of Tropical Medicine, Antwerp; L. Vandekerckhove, University Ziekenhuis Gent, Gent. Bosnia-Herzegovina: (V. Hadziosmanovic), Klinicki Centar Univerziteta Sarajevo, Sarajevo. Bulgaria: (K. Kostov), Infectious Diseases Hospital, Sofia. Croatia: (J. Begovac), University Hospital of Infectious Diseases, Zagreb. Czech Republic: (L. Machala), D. Jilich, Faculty Hospital Bulovka, Prague; D. Sedlacek, Charles University Hospital, Plzen. Denmark: (J. Nielsen), G. Kronborg, T. Benfield, M. Larsen, Hvidovre Hospital, Copenhagen; J. Gerstoft, T. Katzenstein, A.-B.E. Hansen, P. Skinhøj, Rigshospitalet, Copenhagen; C. Pedersen, Odense University Hospital, Odense; L. Ostergaard, Skejby Hospital, Aarhus. Estonia: (K. Zilmer), West-Tallinn Central Hospital, Tallinn; Jelena Smidt, Nakkusosakond Siseklinik, Kohtla-Järve. Finland: (M. Ristola), Helsinki University Central Hospital, Helsinki. France: (C. Katlama), Hôpital de la Pitié-Salpétière, Paris; J.-P. Viard, Hôpital Necker-Enfants Malades, Paris; P.-M. Girard, Hospital Saint-Antoine, Paris; J.M. Livrozet, Hôpital Edouard Herriot, Lyon; P. Vanhems, University Claude Bernard, Lyon; C. Pradier, Hôpital de l'Archet, Nice; F. Dabis*, D. Neau, Unité INSERM, Bordeaux. Germany: (J. Rockstroh), Universitäts Klinik Bonn; R. Schmidt, Medizinische Hochschule Hannover; J. van Lunzen, O. Degen, University Medical Center Hamburg-Eppendorf, Infectious Diseases Unit, Hamburg; H.J. Stellbrink, IPM Study Center, Hamburg; S. Staszewski, J.W. Goethe University Hospital, Frankfurt; M. Bickel, Medizinische Poliklinik, Munich; G. Fätkenheuer, Universität Köln, Cologne. Greece: (J. Kosmidis), P. Gargalianos, G. Xylomenos, J. Perdios, Athens General Hospital; G. Panos, A. Filandras, E. Karabatsaki, 1st IKA Hospital; H. Sambatakou, Ippokration Genereal Hospital, Athens. Hungary: (D. Banhegyi), Szent Lásló Hospital, Budapest. Ireland: (F. Mulcahy), St. James's Hospital, Dublin. Israel: (I. Yust), D. Turner, M. Burke, Ichilov Hospital, Tel Aviv; S. Pollack, G. Hassoun, Rambam Medical Center, Haifa; S. Maayan, Hadassah University Hospital, Jerusalem. Italy: (S. Vella), Istituto Superiore di Sanità, Rome; R. Esposito, I. Mazeu, C. Mussini, Università Modena, Modena; C. Arici, Ospedale Riuniti, Bergamo; R. Pristera, Ospedale Generale Regionale, Bolzano; F. Mazzotta, A. Gabbuti, Ospedale S. Maria Annunziata, Firenze; V. Vullo, M. Lichtner, University di Roma la Sapienza, Rome; A. Chirianni, E. Montesarchio, M. Gargiulo, Presidio Ospedaliero A.D. Cotugno, Monaldi Hospital, Napoli; G. Antonucci, A. Testa, P. Narciso, C. Vlassi, M. Zaccarelli, Istituto Nazionale Malattie Infettive Lazzaro Spallanzani, Rome; A. Lazzarin, A. Castagna, N. Gianotti, Ospedale San Raffaele, Milan; M. Galli, A. Ridolfo, Osp. L. Sacco, Milan; A. d'Arminio Monforte, Istituto Di Clinica Malattie Infettive e Tropicale, Milan. Latvia: (B. Rozentale), I. Zeltina, Infectology Centre of Latvia, Riga. Lithuania: (S. Chaplinskas), Lithuanian AIDS Centre, Vilnius. Luxembourg: (R. Hemmer), T. Staub, Centre Hospitalier, Luxembourg. Netherlands: (P. Reiss*), Academisch Medisch Centrum bij de Universiteit van Amsterdam, Amsterdam. Norway: (V. Ormaasen), A. Maeland, J. Bruun, Ullevål Hospital, Oslo. Poland: (B. Knysz), J. Gasiorowski, Medical University, Wroclaw; A. Horban, E. Bakowska, Centrum Diagnostyki i Terapii AIDS, Warsaw; A. Grzeszczuk, R. Flisiak, Medical University, Bialystok; A. Boron-Kaczmarska, M. Pynka, M. Parczewski, Medical Univesity, Szczecin; M. Beniowski, E. Mularska, Osrodek Diagnostyki i Terapii AIDS, Chorzow; H. Trocha, Medical University, Gdansk; E. Jablonowska, E. Malolepsza, K. Wojcik, Wojewodzki Szpital Specjalistyczny, Lodz. Portugal: (F. Antunes), M. Doroana, L. Caldeira, Hospital Santa Maria, Lisbon; K. Mansinho, Hospital de Egas Moniz, Lisbon; F. Maltez, Hospital Curry Cabral, Lisbon. Romania: (D. Duiculescu), Spitalul de Boli Infectioase si Tropicale: Dr Victor Babes, Bucarest. Russia: (A. Rakhmanova), Medical Academy Botkin Hospital, St Petersburg; N. Zakharova, St Petersburg AIDS Centre, St Peterburg; S. Buzunova, Novgorod Centre for AIDS, Novgorod. Serbia: (D. Jevtovic), The Institute for Infectious and Tropical Diseases, Belgrade. Slovakia: (M. Mokráš), D. Staneková, Dérer Hospital, Bratislava. Slovenia: (J. Tomazic), University Clinical Centre Ljubljana, Ljubljana. Spain: (J. González-Lahoz), V. Soriano, P. Labarga, J. Medrano, Hospital Carlos III, Madrid; S. Moreno, J.M. Rodriguez, Hospital Ramon y Cajal, Madrid; B. Clotet, A. Jou, R. Paredes, C. Tural, J. Puig, I. Bravo, Hospital Germans Trias i Pujol, Badalona; J.M. Gatell, J.M. Miró, Hospital Clinic i Provincial, Barcelona; P. Domingo, M. Gutierrez, G. Mateo, M.A. Sambeat, Hospital Sant Pau, Barcelona. Sweden: (A. Karlsson), Venhaelsan-Sodersjukhuset, Stockholm; L. Flamholc, Malmö University Hospital, Malmö. Switzerland: (B. Ledergerber), R. Weber*, University Hospital, Zürich; P. Francioli, M. Cavassini, Centre Hospitalier Universitaire Vaudois, Lausanne; B. Hirschel, E. Boffi, Hospital Cantonal Universitaire de Geneve, Geneve; H. Furrer, Inselspital Bern, Bern; M. Battegay, L. Elzi, University Hospital Basel. Ukraine: (E. Kravchenko), N. Chentsova, Kiev Centre for AIDS, Kiev; V. Frolov, G. Kutsyna, Luhansk State Medical University; Luhansk; S. Servitskiy, Odessa Region AIDS Center, Odessa; M. Krasnov, Kharkov State Medical University, Kharkov. United Kingdom: (S. Barton), St. Stephen's Clinic, Chelsea and Westminster Hospital, London; A.M. Johnson, D. Mercey, Royal Free and University College London Medical School, London (University College Campus); A. Phillips, M.A. Johnson, A. Mocroft, Royal Free and University College Medical School, London (Royal Free Campus); M. Murphy, Medical College of Saint Bartholomew's Hospital, London; J. Weber, G.

Scullard, Imperial College School of Medicine at St. Mary's, London; M. Fisher, Royal Sussex County Hospital, Brighton; C. Leen, Western General Hospital, Edinburgh.

HivBivus (Sweden): Central coordination: L. Morfeldt*, G. Thulin, A. Sundström. Participating physicians (city): B. Åkerlund (Huddinge); K. Koppel, A. Karlsson (Stockholm); L. Flamholc, C. Håkangård (Malmö).

The ICONA Foundation (Italy): Board of directors: M. Moroni (Chair), G. Angarano, A. Antinori, O. Armignacco, A. d'Arminio Monforte*, F. Castelli, R. Cauda, G. Di Perri, M. Galli, R. Iardino, G. Ippolito, A. Lazzarin, C.F. Perno, F. von Schloesser, P. Viale Scientific secretary: A d'Arminio Monforte*, A. Antinori, A. Castagna, F. Ceccherini-Silberstein, A. Cozzi-Lepri, E. Girardi, S. Lo Caputo, C. Mussini, M. Puoti ICONA Steering Committee: M. Andreoni, A. Ammassari, A. Antinori, A. d'Arminio Monforte, C. Balotta, P. Bonfanti, S. Bonora, M. Borderi, R. Capobianchi, A. Castagna, F. Ceccherini-Silberstein, A. Cingolani, P. Cinque, A. Cozzi-Lepri, A. De Luca, A. Di Biagio, E. Girardi, N. Gianotti, A. Gori, G. Guaraldi, G. Lapadula, M. Lichtner, S. Lo Caputo, G. Madeddu, F. Maggiolo, G. Marchetti, S. Marcotullio, L. Monno, C. Mussini, M. Puoti, E. Quiros Roldan, S. Rusconi. Statistical and monitoring team: A.Cozzi-Lepri, P. Cicconi, I. Fanti, T. Formenti, L. Galli, P. Lorenzini. Participating physicians and centers: A. Giacometti, A. Costantini (Ancona); G. Angarano, L. Monno, C. Santoro (Bari); F. Maggiolo, C. Suardi (Bergamo); P. Viale, E. Vanino, G. Verucchi (Bologna); F. Castelli, E. Quiros Roldan, C. Minardi (Brescia); T. Quirino, C. Abeli (Busto Arsizio); P.E. Manconi, P. Piano (Cagliari); J. Vecchiet, K. Falasca (Chieti); L. Sighinolfi, D. Segala (Ferrara); F. Mazzotta, S. Lo Caputo (Firenze); G. Cassola, G. Viscoli, A. Alessandrini, R. Piscopo, G. Mazzarello (Genova); C. Mastroianni, V. Belvisi (Latina); P. Bonfanti, I. Caramma (Lecco); A. P. Castelli (Macerata); M. Galli, A. Lazzarin, G. Rizzardini, M. Puoti, A. d'Arminio Monforte, A.L. Ridolfo, R. Piolini, A. Castagna, S. Salpietro, L. Carenzi, M.C. Moioli, P. Cicconi, G. Marchetti (Milano); C. Mussini, C. Puzzolante (Modena); A. Gori, G. Lapadula (Monza); N. Abrescia, A. Chirianni, M.G. Guida, M. ONOFRIO (Napoli); F. Baldelli, D. Francisci (Perugia); G. Parruti, T. Ursini (Pescara); G. Magnani, M.A. Ursitti (Reggio Emilia); R. Cauda, M. Andreoni, A. Antinori, V. Vullo, A. Cingolani, A. d'Avino, A. Ammassari, L. Gallo, E. Nicastri, R. Acinapura, M. Capozzi, R. Libertone, G. Tebano (Roma); A. Cattelan (Rovigo); M.S. Mura, G. Madeddu (Sassari); P. Caramello, G. Di Perri, G.C. Orofino, S. Bonora, M. Sciandra (Torino); G. Pellizzer, V. Manfrin (Vicenza).

Nice HIV Cohort (France): Central coordination: C. Pradier*, E. Fontas, C. Caissotti. Participating physicians: P. Dellamonica, E. Bernard, E. Cua, F. De Salvador-Guillouet, J. Durant, S. Ferrando, V. Mondain-Miton, A. Naqvi, I. Perbost, B. Prouvost-Keller, S. Pillet, P. Pugliese, V. Rahelinirina, P.M. Roger. Clinical research assistant: K. Dollet.

SHCS (Swiss HIV Cohort Study, Switzerland): V. Aubert, M. Battegay, E. Bernasconi, J. Böni, H.C. Bucher, C. Burton-Jeangros, A. Calmy, M. Cavassini, G. Dollenmaier, M. Egger, L. Elzi, J. Fehr, J. Fellay, H. Furrer (Chairman of the Clinical and Laboratory Committee), C.A. Fux, M. Gorgievski, H. Günthard (President of the SHCS), D. Haerry (deputy of 'Positive Council'), B. Hasse, H.H. Hirsch, M. Hoffmann, I. Hösli, C. Kahlert, L. Kaiser, O. Keiser, T. Klimkait, R. Kouyos, H. Kovari, B. Ledergerber, G. Martinetti, B. Martinez de Tejada, K. Metzner, N. Müller, D. Nadal, D. Nicca, G. Pantaleo, A. Rauch (Chairman of the Scientific Board), S. Regenass, M. Rickenbach (Head of Data Center), C. Rudin (Chairman of the Mother and Child Substudy), F. Schöni-Affolter, P. Schmid, J. Schüpbach, R. Speck, P. Tarr, A. Telenti, A. Trkola, P. Vernazza, R. Weber*, S. Yerly.

The data are gathered by the Five Swiss University Hospitals, two Cantonal Hospitals, 15 affiliated hospitals and 36 private physicians (listed in http://www.shcs.ch/31-health-care-providers).

This project was presented at the CROI, 3–6 March 2014, Boston, Massachusetts, USA, abstract # 1289.

Ethics committee approval: This analysis was conducted in accordance with the Declaration of Helsinki and approved by national ethical committee where necessary.

The content of this publication is solely the responsibility of the authors and does not necessarily represent the official views of any of the institutions mentioned above.

Conflicts of interest

Author contributions: L.R., J.D.L. and C.S. developed the initial analysis protocol. L.R. performed study coordination and prepared the datasets for analysis, and C.S. performed the statistical analysis. L.R. prepared the first draft of the manuscript. All authors have provided input at all stages of the project.

L.R., J.D.L., W.E.S., S.D.W., F.D. and E.F. have no conflicts of interest. P.R. has served as a scientific advisor to Bristol-Myers Squibb, Gilead Sciences, Grupo Ferrer, GlaxoSmithKline, Janssen Pharmaceuticals, Merck, Inc and ViiV Healthcare. He has served on data and safety monitoring boards and endpoint adjudication committees for Janssen Pharmaceuticals and his institution has received honoraria for speaking engagements at scientific conferences from Bristol-Myers Squibb, Gilead Sciences, Inc, and GlaxoSmithKline. He has received research support from Gilead Sciences, ViiV Healthcare, Merck,

Inc, Janssen Pharmaceuticals, Bristol-Myers Squibb, Abbott and Boehringer Ingelheim. M.L. has received research grants from Boehringer Ingelheim, Bristol Myer Squibb, Gilead Sciences, GlaxoSmithKline, Janssen Pharmaceuticals, Merck, Pfizer and Hoffman-LaRoche. A.M. has received consultancy fees/honoraria/speaker fees from Bristol-Myers Squibb, Pfizer, Merck, Boehringer Ingelheim and Gilead Sciences. H.K. has in the past received consultancy and grants paid to her institution by Gilead Sciences and travel expenses/accommodation/meeting expenses paid by Gilead Sciences and Bristol-Myers Squibb. A.D.M. has past board membership at Abbvie, Bristol-Myers Squibb, Gilead Sciences, Janssen Pharmaceuticals and Merck. C.S. has a pending grant from Bristol-Myers Squibb and received payment for development of educational presentations by Gilead Sciences, ViiV Healthcare and Janssen Pharmaceuticals. A.P. received personal fees from Gilead Sciences, Abbvie and GlaxoSmithKline Vaccines and grants from Bristol-Myers Squibb. C.S. received personal fees from Gilead Sciences, Bristol-Myers Squibb, Janssen Pharmaceuticals, Abbott Pharmaceuticals and ViiV Healthcare.

References

1. Wit FW, Weverling GJ, Weel J, Jurriaans S, Lange JM. **Incidence of and risk factors for severe hepatotoxicity associated with antiretroviral combination therapy.** *J Infect Dis* 2002; **186**:23–31.
2. Kovari H, Weber R. **Influence of antiretroviral therapy on liver disease.** *Curr Opin HIV AIDS* 2011; **6**:272–277.
3. Abrescia N, D'Abbraccio M, Figoni M, Busto A, Maddaloni A, De Marco M. **Hepatotoxicity of antiretroviral drugs.** *Curr Pharm Des* 2005; **11**:3697–3710.
4. European AIDS Clinical Society. EACS Treatment Guidelines version 8.0; 2015. http://www.eacsociety.org/guidelines/eacs-guidelines/eacs-guidelines.html. [Accessed 5 November 2015]
5. Maida I, Nunez M, Rios MJ, Martin-Carbonero L, Sotgiu G, Toro C, *et al*. **Severe liver disease associated with prolonged exposure to antiretroviral drugs.** *J Acquir Immune Defic Syndr* 2006; **42**:177–182.
6. Kalyesubula R, Kagimu M, Opio KC, Kiguba R, Semitala CF, Schlech WF, *et al*. **Hepatotoxicity from first line antiretroviral therapy: an experience from a resource limited setting.** *Afr Health Sci* 2011; **11**:16–23.
7. Sulkowski MS, Thomas DL, Chaisson RE, Moore RD. **Hepatotoxicity associated with antiretroviral therapy in adults infected with human immunodeficiency virus and the role of hepatitis C or B virus infection.** *JAMA* 2000; **283**:74–80.
8. Lapadula G, Costarelli S, Chatenoud L, Castelli F, Astuti N, Di Giambenedetto S, *et al*. **Risk of liver enzyme elevation during treatment with ritonavir-boosted protease inhibitors among HIV-monoinfected and HIV/HCV coinfected patients.** *J Acquir Immune Defic Syndr* 2015; **69**:312–318.
9. Kovari H, Ledergerber B, Battegay M, Rauch A, Hirschel B, Foguena AK, *et al*. **Incidence and risk factors for chronic elevation of alanine aminotransferase levels in HIV-infected persons without hepatitis B or C virus co-infection.** *Clin Infect Dis* 2010; **50**:502–511.
10. Peters L, Rockstroh JK. **Biomarkers of fibrosis and impaired liver function in chronic hepatitis C: how well do they predict clinical outcomes?** *Curr Opin HIV AIDS* 2010; **5**:517–523.
11. Suarez-Zarracina T, Valle-Garay E, Collazos J, Montes AH, Carcaba V, Carton JA, *et al*. **Didanosine (ddI) associates with increased liver fibrosis in adult HIV-HCV coinfected patients.** *J Viral Hepat* 2012; **19**:685–693.
12. Pinol V, Bessa X, Bruguera M, Rodes J. **Steatosis and nonalcoholic steatohepatitis. A comparative analysis.** *Gastroenterol Hepatol* 2000; **23**:57–61.
13. Loko MA, Bani-Sadr F, Winnock M, Lacombe K, Carrieri P, Neau D, *et al*. **Impact of HAART exposure and associated lipodystrophy on advanced liver fibrosis in HIV/HCV-coinfected patients.** *J Viral Hepat* 2011; **18**:e307–e314.
14. Blanco F, Barreiro P, Ryan P, Vispo E, Martin-Carbonero L, Tuma P, *et al*. **Risk factors for advanced liver fibrosis in HIV-infected individuals: role of antiretroviral drugs and insulin resistance.** *J Viral Hepat* 2011; **18**:11–16.
15. Macias J, Berenguer J, Japon M, *et al*. **Cumulative exposure to ARV drugs leads to progressive hepatic steatosis among HIV/HCV-co-infected patients [Abstract 781].** *19th CROI. Seattle, Washington, USA;* 5–8 March 2012.
16. Bani-Sadr F, Lapidus N, Bedossa P, De Boever CM, Perronne C, Halfon P, *et al*. **Progression of fibrosis in HIV and hepatitis C virus-coinfected patients treated with interferon plus ribavirin-based therapy: analysis of risk factors.** *Clin Infect Dis* 2008; **46**:768–774.
17. McGovern BH, Ditelberg JS, Taylor LE, Gandhi RT, Christopoulos KA, Chapman S, *et al*. **Hepatic steatosis is associated with fibrosis, nucleoside analogue use, and hepatitis C virus genotype 3 infection in HIV-seropositive patients.** *Clin Infect Dis* 2006; **43**:365–372.
18. Merchante N, Perez-Camacho I, Mira JA, Rivero A, Macias J, Camacho A, *et al*. **Prevalence and risk factors for abnormal liver stiffness in HIV-infected patients without viral hepatitis coinfection: role of didanosine.** *Antivir Ther* 2010; **15**:753–763.
19. Borghi V, Puoti M, Mussini C, Bellelli S, Angeletti C, Sabbatini F, *et al*. **HIV coinfection and antiretroviral therapy enhances liver steatosis in patients with hepatitis C, but only in those infected by HCV genotype other than 3.** *Antivir Ther* 2008; **13**:1057–1065.
20. Kovari H, Sabin CA, Ledergerber B, Ryom L, Worm SW, Smith C, *et al*. **Antiretroviral drug-related liver mortality among HIV-positive persons in the absence of hepatitis B or C virus coinfection: the data collection on adverse events of anti-HIV drugs study.** *Clin Infect Dis* 2013; **56**:870–879.
21. Mocroft A, Soriano V, Rockstroh J, Reiss P, Kirk O, de Wit S, *et al*. **Is there evidence for an increase in the death rate from liver-related disease in patients with HIV?** *AIDS* 2005; **19**:2117–2125.
22. Marine-Barjoan E, Saint-Paul MC, Pradier C, Chaillou S, Anty R, Michiels JF, *et al*. **Impact of antiretroviral treatment on progression of hepatic fibrosis in HIV/hepatitis C virus co-infected patients.** *AIDS* 2004; **18**:2163–2170.
23. Benhamou Y, Di Martino V, Bochet M, Colombet G, Thibault V, Liou A, *et al*. **Factors affecting liver fibrosis in human immunodeficiency virus-and hepatitis C virus-coinfected patients: impact of protease inhibitor therapy.** *Hepatology* 2001; **34**:283–287.
24. Weber R, Sabin CA, Friis-Moller N, Reiss P, El-Sadr WM, Kirk O, *et al*. **Liver-related deaths in persons infected with the human immunodeficiency virus: the D:A:D study.** *Arch Intern Med* 2006; **166**:1632–1641.
25. Qurishi N, Kreuzberg C, Luchters G, Effenberger W, Kupfer B, Sauerbruch T, *et al*. **Effect of antiretroviral therapy on liver-related mortality in patients with HIV and hepatitis C virus coinfection.** *Lancet* 2003; **362**:1708–1713.
26. Shubber Z, Calmy A, Andrieux-Meyer I, Vitoria M, Renaud-Thery F, Shaffer N, *et al*. **Adverse events associated with nevirapine and efavirenz-based first-line antiretroviral therapy: a systematic review and meta-analysis.** *AIDS* 2013; **27**:1403–1412.
27. Phan V, Thai S, Choun K, Lynen L, van Griensven J. **Incidence of treatment-limiting toxicity with stavudine-based antiretroviral therapy in Cambodia: a retrospective cohort study.** *PLoS One* 2012; **7**:e30647.
28. Pascual Pareja JF, Camino A, Larrauri J, Lopez-Dieguez M, Montes ML, Gonzalez-Garcia J, *et al*. **Factors associated with hepatic steatosis in human immunodeficiency virus and hepatits C virus coinfected patients.** *Med Clin (Barc)* 2009; **132**:208–213.
29. Miller KD, Cameron M, Wood LV, Dalakas MC, Kovacs JA. **Lactic acidosis and hepatic steatosis associated with use of stavudine: report of four cases.** *Ann Intern Med* 2000; **133**:192–196.

30. Bleeker-Rovers CP, Kadir SW, van Leusen R, Richter C. **Hepatic steatosis and lactic acidosis caused by stavudine in an HIV-infected patient.** *Neth J Med* 2000; **57**:190–193.
31. Bekolo CE, Sonkoue C, Djidjou H, Bekoule PS, Kollo B. **Evaluating the utility of early laboratory monitoring of antiretroviral-induced haematological and hepatic toxicity in HIV-infected persons in Cameroon.** *BMC Infect Dis* 2014; **14**:519.
32. Kovari H, Ledergerber B, Peter U, Flepp M, Jost J, Schmid P, *et al*. **Association of noncirrhotic portal hypertension in HIV-infected persons and antiretroviral therapy with didanosine: a nested case-control study.** *Clin Infect Dis* 2009; **49**:626–635.
33. Vispo E, Moreno A, Maida I, Barreiro P, Cuevas A, Albertos S, *et al*. **Noncirrhotic portal hypertension in HIV-infected patients: unique clinical and pathological findings.** *AIDS* 2010; **24**:1171–1176.
34. Scourfield A, Jackson A, Waters L, Gazzard B, Nelson M. **The value of screening HIV-infected individuals for didanosine-related liver disease?** *Antivir Ther* 2011; **16**:941–942.
35. WHO. March 2014 supplement to the 2013 consolidated guidelines on the use of antiretroviral drugs for treating and preventing HIV infection. Recommendations for a public health approach; 2014. http://www.who.int/hiv/pub/guidelines/arv2013/arvs2013upplement_march2014/en/. [Accessed 14 September 2015]
36. Podlekareva D, Grint D, Karpov I, Rakmanova A, Mansinho K, Chentsova N, *et al*. **Changing utilization of Stavudine (D4T) in HIV-positive people in 2006-2013 in the EuroSIDA cohort.** *HIV Med* 2015; **16**:533–543.
37. Macias J, Neukam K, Mallolas J, Lopez-Cortes LF, Carton JA, Domingo P, *et al*. **Liver toxicity of initial antiretroviral drug regimens including two nucleoside analogs plus one non-nucleoside analog or one ritonavir-boosted protease inhibitor in HIV/HCV-coinfected patients.** *HIV Clin Trials* 2012; **13**:61–69.
38. Chu KM, Boulle AM, Ford N, Goemaere E, Asselman V, Van Cutsem G. **Nevirapine-associated early hepatotoxicity: incidence, risk factors, and associated mortality in a primary care ART programme in South Africa.** *PLoS One* 2010; **5**:e9183.
39. Dieterich DT, Robinson PA, Love J, Stern JO. **Drug-induced liver injury associated with the use of nonnucleoside reverse-transcriptase inhibitors.** *Clin Infect Dis* 2004; **38 (Suppl 2)**:S80–S89.
40. Vogel M, Rockstroh JK. **Hepatotoxicity and liver disease in the context of HIV therapy.** *Curr Opin HIV AIDS* 2007; **2**:306–313.
41. Mikl J, Sulkowski MS, Benhamou Y, Dieterich D, Pol S, Rockstroh J, *et al*. **Hepatic profile analyses of tipranavir in Phase II and III clinical trials.** *BMC Infect Dis* 2009; **9**:203.
42. Spagnuolo V, Gentilini G, De Bona A, Galli L, Uberti-Foppa C, Soldarini A, *et al*. **Liver function parameters in HIV/HCV co-infected patients treated with amprenavir and ritonavir and correlation with plasma levels.** *New Microbiol* 2007; **30**:279–282.
43. Kowalska JD, Friis-Moller N, Kirk O, Bannister W, Mocroft A, Sabin C, *et al*. **The coding causes of death in HIV (CoDe) project: initial results and evaluation of methodology.** *Epidemiology* 2011; **22**:516–523.
44. Friis-Moller N, Sabin CA, Weber R, d'Arminio Monforte A, El-Sadr WM, Reiss P, *et al*. **Combination antiretroviral therapy and the risk of myocardial infarction.** *N Engl J Med* 2003; **349**:1993–2003.
45. Lo Re V 3rd, Kallan MJ, Tate JP, Localio AR, Lim JK, Goetz MB, *et al*. **Hepatic decompensation in antiretroviral-treated patients co-infected with HIV and hepatitis C virus compared with hepatitis C virus-monoinfected patients: a cohort study.** *Ann Intern Med* 2014; **160**:369–379.
46. Lo RV, Kallan M, Tate J, Localio R, Lim J, Goetz M, *et al*. **Determinants of hepatic decompensation in ARV-treated HIV/hepatitis C virus co-infected patients: Atlanta, Georgia, USA.** *CROI,* 2013.
47. Moyle G. **Toxicity of antiretroviral nucleoside and nucleotide analogues: is mitochondrial toxicity the only mechanism?** *Drug Saf* 2000; **23**:467–481.
48. Walker UA, Bauerle J, Laguno M, Murillas J, Mauss S, Schmutz G, *et al*. **Depletion of mitochondrial DNA in liver under antiretroviral therapy with didanosine, stavudine, or zalcitabine.** *Hepatology* 2004; **39**:311–317.
49. Kosi L, Reiberger T, Payer BA, Grabmeier-Pfistershammer K, Strassl R, Rieger A, *et al*. **Five-year on-treatment efficacy of lamivudine-, tenofovir- and tenofovir + emtricitabine-based HAART in HBV-HIV-coinfected patients.** *J Viral Hepat* 2012; **19**:801–810.
50. Kovari H, Sabin C, Ledergerber B, Ryom L, Monforte A, Law M, *et al*. **Antiretroviral drugs associated with chronic ALT elevations in persons withouy HCV and HBV infection: Seattle, Washington, USA.** *CROI,* 2015.
51. Qayyum S, Dong H, Kovacic D, Sohail S, Waters B, Thornton C, *et al*. **Combination therapy efavirenz/emtricitabine/tenofovir disoproxil fumarate associated with hepatic failure.** *Curr Drug Saf* 2012; **7**:391–393.
52. Lattuada E, Lanzafame M, Carolo G, Gottardi M, Concia E, Vento S. **Does tenofovir increase efavirenz hepatotoxicity?** *AIDS* 2008; **22**:995.
53. Kohler JJ, Hosseini SH, Hoying-Brandt A, Green E, Johnson DM, Russ R, *et al*. **Tenofovir renal toxicity targets mitochondria of renal proximal tubules.** *Lab Invest* 2009; **89**:513–519.
54. FDA. Drug Approval Package VIREAD (Tenofovir Disoproxil Fumarate) Tablets; 2001. http://www.accessdata.fda.gov/drugsatfda_docs/nda/2001/21–356_Viread.cfm. [Accessed 14 September 2015]
55. Wood R, Gathe JC, Givens N, Sedani S, Cheng K, Sievers J. **Long-term safety study of fosamprenavir-containing regimens in HIV-1-infected patients.** *HIV Clin Trials* 2013; **14**:183–191.
56. Amprenavir package leaflet. Summary of Product Characteristics. http://www.ema.europa.eu/docs/en_GB/document_library/EPAR_-_Product_Information/human/000264/WC500022929.pdf. [Accessed 14 September 2015]
57. Esposito V, Verdina A, Manente L, Spugnini EP, Viglietti R, Parrella R, *et al*. **Amprenavir inhibits the migration in human hepatocarcinoma cell and the growth of xenografts.** *J Cell Physiol* 2013; **228**:640–645.
58. Seminari E, De Bona A, Gentilini G, Galli L, Schira G, Gianotti N, *et al*. **Amprenavir and ritonavir plasma concentrations in HIV-infected patients treated with fosamprenavir/ritonavir with various degrees of liver impairment.** *J Antimicrob Chemother* 2007; **60**:831–836.